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Commentary

(Special Topic)

## Insecticide resistance through mutations in cholinesterases or carboxylesterases: data mining in the ESTHER database

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Resistance of arthropods to organophosphates and carbamates used as insecticides is mainly due to mutations in genes encoding carboxylesterase or acetylcholinesterase members of the alpha/beta-hydrolase fold superfamily of proteins. Mutations that have been described at the molecular level concern 24 species, 31 genes and 32 identical positions in the aligned aminoacid sequences. Seven of these positions are found in more than four species and can be considered as hot spots for mutations. Mutations in one single gene also result in cross resistance to pyrethroids. These figures along with all pieces of information related to these mutations can be recovered from the ESTHER database, dedicated to the alpha/beta-hydrolase fold superfamily (<http://bioweb.ensam.inra.fr/esther>), through built-in or custom made queries. A sequence alignment of enzymes involved in resistance with highlighted mutated amino acid residues is provided. Selecting one amino acid residue leads to all information about mutations analyzed at this position. Links to the related literature are also available. © Pesticide Science Society of Japan

**Keywords:** acetylcholinesterase, alpha/beta-hydrolase fold, carbamate, carboxylesterase, cholinesterase, ESTHER database, insecticide resistance, mutation, organophosphate.

### Introduction

The analysis of origin and molecular mechanisms of resistance to pesticides by arthropods is a very active field of research. Most insecticides act on the nervous system at synaptic sites. Organophosphates (OPs) and carbamates, which inhibit acetylcholinesterase (AChE) and prolong the excitatory action of acetylcholine (ACh), are still the dominant insecticides in terms of number and quantities of compounds used, and market value.<sup>1)</sup> In the case of resistance to OPs and carbamates, the activity of unspecific detoxification enzymes such as glutathione-*S*-transferase or mixed-function

oxidases is often increased in resistant pests, although most of the proteins involved in strain adaptation belong to the carboxylesterase or cholinesterase families.<sup>2–4)</sup> Genome modifications that give rise to resistance are very diverse and include: point mutations in the genes encoding the insecticide targets, amplification of esterase genes resulting in over-expressed proteins that sequester the insecticides, mutations that transform a carboxylesterase into an OP hydrolase, and amplification of a resistant AChE gene. However all these modifications do not generate a same level of resistance and fitness cost. Comparative analysis of the numerous experiments and species concerned by this mode of selective pressure are necessary. Only a database compilation strategy can retrieve this overwhelming flow of information in a structured way. The ESTHER database, which was created 15 years ago, gathers and annotates all the published information related to members of the alpha/beta-hydrolase fold along with bio-

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**Table 1.** Genes, mutations, species, Torpedo\_number and references displayed in this table were gathered as a table from a query available at : <http://bioweb.ensam.inra.fr/ESTHER/general?what=aqlinsectresist>.

Gene/Taxon	Species	Enzyme	Torpedo_number	Mutations	Ref.
<i>ace1</i>					
Diptera					
	<i>Anopheles gambiae</i>	anoga-acche1	119	G119S	13–16
	<i>Culex pipiens</i>	culpi-acche1	119, 290	G119S, F416V	17–21
	<i>Culex tritaeniorhynchus</i>	cultr-acche1	331	F455W	17, 23
Lepidoptera					
	<i>Cydia pomonella</i>	cydpo-acche1	290	F399V	24
	<i>Chilo suppressalis</i>	9neop-acche1	201	A314S	25
	<i>Plutella xylostella</i>	pluxy-acche1	131, 201, 227, 441	D131G, A201S, G227A, A441G	26,27
Hemiptera					
	<i>Aphis gossypii</i>	aphgo-acche1	201, 331	A302S/S431F, S431F	28–32
	<i>Bemisia tabaci</i>	bemta-acche1	331	F392W	33
	<i>Myzus persicae</i>	myzpe-acchem	331	S431F	34–37
	<i>Rhopalosiphum padi</i>	rhodp-acche1	228	S329P	38
	<i>Sitobion avenae</i>	sitav-acche1	336	L436S	39
Arachnida Acari					
	<i>Boophilus microplus</i>	boomi-acche1	131, 140, 275, 336	D188G/E196G/V331A /F390S	40
	<i>Tetranychus kanzawai</i>	tetka-acche	331	F439W	41
	<i>Tetranychus urticae</i>	tetur-acche	201, 280, 328, 331	A201S, T280A, G328A, F439C/W/Y	42–44
<i>ace2</i>					
Diptera					
	<i>Aedes aegypti</i>	aedae-acche	78, 227, 288	F105S, G285A, F350Y	45
	<i>Bactrocera dorsalis</i>	bacdo-acche	129, 396	I214V/G488S/Q643R	46
	<i>Bactrocera oleae</i>	bacol-acche	129, 396, 551, 552, 553, 554	I214V, G488S, Q642-644del	47–51
	<i>Ceratitis capitata</i>	cerca-acche	328	G328A	52
	<i>Drosophila melanogaster</i>	drome-acche	70, 72, 74, 78, 84, 121, 129, 130, 199, 227, 276, 279, 284, 288, 290, 328, 330, 331, 334, 335	E107+A, E107A, E107o, Y109A, Y111A, F115S, W121A, W121Y, M191A, I199A/E/G/K/R/T/W, Y200A, E275A, E275Q, G303A, V356A, W359+A, W359A, W359o, Y362A, L366A, L366F, F368A, F368C/H/I/L/S/V/W/Y, G406A, Y408A/F, F409A, Y412+A, Y412A, Y412o, D413A,	53–59
	<i>Haematobia irritans</i>	haeir-acche	227	G262A	60
	<i>Lucilia cuprina</i>	luccu-acche	78,78,129,227,290	F115S, I199T/V, G303A, F368Y	59

**Table 1.** Continued.

Gene/Taxon	Species	Enzyme	Torpedo_number	Mutations	Ref.
Coleoptera	<i>Musca domestica</i>	musdo-acche	83, 150, 195, 227, 290, 304, 32	I82M, V180L, T230M, G262A/V, F327Y, D341L, G365A, A586T	61–68
	<i>Leptinotarsa decemlineata</i>	lepde-acche	–6, 238, 337	R30K, S291G, I392T	69–74
Hemiptera	<i>Aphis gossypii</i>	aphgo-acche2	78	F139L	28,30
	<i>Rhopalosiphum padi</i>	rhpd-acche2	290, 356	F368L, V435	38
	<i>Sitobion avenae</i>	sitav-acche2	435	W516R	39
<i>ace3</i>					
Arachnida Acari	<i>Boophilus microplus</i>	boomi-acche3		R86Q	75, 76
Carboxylesterase					
Diptera	<i>Cochliomyia hominivorax</i>	cocho-E3aest7	119, 233	G137D, W251S	77–80
	<i>Drosophila melanogaster</i>	drome-EST23aes07	119, 233	G137D, W251L	81
	<i>Lucilia cuprina</i>	luccu-E3aest7	119, 130, 233, 199, 232, 233, 290, 330	G137D/E/H/R, Y148F, E217M, W251A/G/L/S/T, F309L, F354/W	81–87
Hymenoptera	<i>Musca domestica</i>	musdo-EST23aes07	119, 233	G137D, W251S, W251C	88–92
	<i>Anisopteromalus calandrae</i>	anica-cxest	233	W220G	93
Hemiptera	<i>Aphis gossypii</i>	aphgo-cxest	17, 376	K14Q/N354D	94
Arachnida	<i>Boophilus microplus</i>	boomi-este13	346	D374N	95

chemical, pharmacological and structural data.<sup>5–10</sup> The carboxylesterase/cholinesterase family (PF00135 COesterase in PFAM database<sup>11</sup>) evolved from a core alpha/beta-hydrolase.<sup>12</sup> Members of this family are numerous in the fungi/metazoa lineage and occupy a prominent place in the ESTHER database. More recently, various tools were developed and included in the database for analysis of natural or directed mutations in members of the alpha/beta-hydrolases, and more specifically the carboxylesterases and cholinesterases.<sup>8–10</sup>

### Alignments of Mutated Sequences

The Mutalign tool displays an alignment of cholinesterases where mutated amino acid residues (either naturally or experimentally) are highlighted and can be selected on a web page. This leads to description of the mutation and its structural/functional effect/impact and bibliographic references can be retrieved. Although equivalent positions in cholinesterase or carboxylesterase sequences from two species may be de-

scribed with different numbers depending on the size of the signal peptide or different insertions/deletions, an international consensus has emerged for using the numbering of *Torpedo californica* AChE as a universal reference. In the ESTHER database this numbering system is referred to as the “Torpedo\_number”. Selecting a Torpedo\_number directs the user towards all the information available on mutations at this particular position in any cholinesterase or carboxylesterase. The original Mutalign presented only the aligned sequences of Torpedo, human, mouse and drosophila AChEs and of human butyrylcholinesterase (i.e., those proteins for which the greatest number of mutations had been studied). We devised a new alignment specifically dedicated to analysis of insecticide resistance. (<http://bioweb.ensam.inra.fr/ESTHER/general?what=mutalignresist>) This alignment includes 34 sequences from 24 species. The *Torpedo californica* AChE sequence is also included to provide access to the Torpedo\_number reference. On top of each column of aligned amino-acid residues, a link represented by a “?” (question mark) leads to the corre-

**Table 2.** Table of genes amplified or duplicated in OP or carbamate resistant insect strains.

ESTHER Gene_locus	Gene	Species	Organism	References
culpi-est2, culpi-est3	Est-2 (EstB), Est-3 (EstA)	<i>Culex pipiens</i> complex	(Mosquito) Diptera	96–99
myzpe-este4, myzpe-estf4	E4 FE4	<i>Myzus persicae</i>	(Peach-potatoe aphid) Hemiptera	100, 101
bemta-coe1	<i>coe1</i>	<i>Bemisia tabaci</i>	(Sweetpotato whitefly) Hemiptera	33
nillu-est1	NI-EST1	<i>Nilaparvata lugens</i>	(Brown planthopper) Hemiptera	102, 103
schga-est1	SG1	<i>Schizaphis graminum</i>	(Greenbug) (Aphid) Hemiptera	104
F290V_culpi-acche1	<i>ace1</i>	<i>Culex pipiens</i>	(Mosquito) Diptera	20
G119S_anoga-acche1	<i>ace1</i>	<i>Anopheles gambiae</i>	(Mosquito) Diptera	105, 106
G228S/A391T/F439W_tetur-acche1	<i>ace1</i>	<i>Tetranychus urticae</i>	(Archnid) Acari	107

sponding Torpedo\_number page with all the mutations studied in this position in any species. The “?” is replaced by a “!” (exclamation mark) when no mutation related to insecticide resistance was described at this particular position while experimental mutations were analyzed in enzymes from other species.

### Complex Queries

The original ACeDB software used to run the database includes both simple queries based on keywords (Simple search, Text search, Class Browser) and more complex queries interconnecting several database objects (Ace Query, AQL Query). Recently a new page was built with different queries related to insecticide resistance. (<http://bioweb.ensam.inra.fr/ESTHER/general?what=aqlinsectresist>). For example one can retrieve a list of publications reporting analysis of natural mutations in carboxylesterases/cholinesterases related to insecticide resistance, with access to all genes and species

```
select p,g,s from m in class Mutation, g in m->Gene_locus, n in m->Modification where n like "OP-*" or n like "ins*" or n like "Pyr*", f in g->Family where f like "AChE" or f like "BuChE" or f like "Carboxylesterase" or f like "Cholin*", s in g->Species, t in s->Tax_id where t like "33208", u in m->Mode_of_mutation where u like "Nat*", p in m->Paper order by :p
```

analyzed in these publications.

#### The Query Is:

The query can be cut and past in the space available in the query page. The tables can be recovered in plain text format and easily introduced in any word processor or tabulator in order to build customized output reports. Table 1 shows a compilation of all mutations related to insecticide resistance in arthropods. The Torpedo\_numbers and related references are included.

Many resistant strains of insects show increased carboxylesterase activity. In some cases the increased enzyme production results not only from increased transcription of the

carboxylesterase genes but also from increased number of copies of those genes in the genome of the resistant insects. Table 2 lists genes that have been amplified or duplicated. Two genes, Est-2 and Est-3, adjacent in the genome of *Culex* mosquitoes, constitute the *Ester* super locus. More than 16 alleles are known at this locus and at least 12 of them are associated with insecticide resistance. Two types of amplification are known, one (A) that co-amplifies Est-2 and Est-3, and one (B) that amplifies only Est-2.<sup>97)</sup> Recently a new allele with Est-3 and Est-2 amplification at a ratio close to 2 : 1 was described.<sup>98)</sup> Amplified carboxylesterase genes were described also in various Hemiptera species. In *Myzus persicae* two adjacent genes are amplified in tandem in some resistant strains.

The size of the amplicon and the different mutations accumulated in the amplified sequence distinguish alleles with different geographical spreading. Number of amplicons, transcription levels of amplified genes and tissue distribution result in various levels of resistance.<sup>97)</sup> Duplication of the AChE target gene was described only for resistant alleles in mosquitoes. Probably only mutated AChE with reduced cholinesterase activity can be amplified without generating a high fitness cost.

### Perspectives

New database classes associated to loci should be introduced in order to include information on haplotypes, intergenic variations, and single nucleotide polymorphism. Since data related to mutations have to be introduced in the database manually from publications, all inputs spontaneously provided by users of the database will be most welcome, and they will be properly acknowledged.

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